IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Group Art Unit 1639

In re

Patent Application of

Allan M. Tereba, et al.

Application No. 10/694,475

Confirmation No.: 4550

Filed: October 27, 2003

Examiner: Christopher M. Gross

"SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA"

Electronically filed by:

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July 17, 2009

Date

APPEAL BRIEF UNDER 37 C.F.R. § 41.37

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This appeal is taken under 35 U.S.C. § 134 and 37 C.F.R. 41.31. Appellants appeal from the non-final Office Action mailed November 18, 2008, in which the Examiner rejected all pending claims. A Notice of Appeal was timely filed on February 17, 2009. The Appeal Brief is accompanied by the fee under 37 C.F.R. 41.20(b)(2), a request for a three-month extension of time, extending the period for reply from April 17, 2009 to July 17, 2009, and by the requisite fee for the extension of time. Please charge or credit Deposit Account No. 50-0842 for any shortage or overpayment of fees associated with this submission.

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REAL PARTY IN INTEREST

The real party in interest is Promega Corporation, the assignee of record.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1-43 are canceled. Claims 44-82 are currently pending; claims 55-57, 59 and 71 are withdrawn from consideration; claims 44-54, 58, 60-70, and 72-82 were rejected in an Office Action mailed November 18, 2008. The rejection of claims 44-54, 58, 60-70, and 72-82 is appealed.

STATUS OF AMENDMENTS

The amendment submitted with the response of February 17, 2009 was entered.

SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 44, and its dependent claims 45-65 and 82, are directed to methods for isolating a defined and consistent amount of DNA from multiple samples (p. 9, lines 21-25; p. 30, line 1-p. 31, line 4) comprising selecting a defined amount of DNA to be isolated from the samples (p. 10, lines 26-30); choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample (p. 6, line 34-p. 7, line 1; p. 17, lines 10-13); contacting each sample with the discrete amount of the silica-containing solid support (p. 6, lines 16-19), each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support (p. 7, lines 7-8), under conditions that allow reversible binding of the defined amount of DNA to the solid support (p. 6, lines 23-24); and separating each sample from the support to isolate a defined and consistent amount of DNA from each sample (p. 6, lines 21-23).

Independent claim 66, and its dependent claims 67, 68, and 77-81, are directed to methods of isolating DNA from multiple samples for use in a molecular biological procedure (p. 9, lines 10-20, and lines 31-33) comprising selecting a defined amount of DNA to be isolated from the samples (p. 5, lines 20-21); choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample (p. 6, lines 34-p. 7, line 1; p. 17, lines 10-13); contacting each sample with a discrete amount of a silica-containing solid support (p. 6, lines 16-19), each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support (p. 7, lines 7-8), under conditions that allow reversible binding of the defined amount of DNA to the solid support (p. 6, lines 23-24); and eluting bound DNA to isolate a defined and consistent amount of DNA from each sample (p. 6, lines 21-23), wherein the eluted DNA is suitable for use in the molecular biological procedure (p. 9, lines 10-20).

Independent claim 69, and its dependent claims 70-76, are directed to kits for isolating a defined and consistent amount of a DNA from multiple samples according to the claimed methods, the kits comprising silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles (p. 7, line 29-p. 8, line 2).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues presented for consideration by the Board are as follows:

1. Whether the Examiner has established a legally sufficient case for rejecting claims 44, 45, 50, 53, 66, 67, and 82 under 35 U.S.C. § 102(b) as being anticipated by Melzak et al. (1996 J. Colloid and Interface Sci. 181:635-644), as evidenced by information available at http://seq.yeastgenome.org/.

2. Whether the Examiner has established a legally sufficient case for rejecting claims 44-54, 58, 60-65, 67, 68, and 77-82 under 35 U.S.C. § 103(a) as being unpatentable over Melzak et al., in view of Kleiber et al. (WO 96/41811).

3. Whether the Examiner has established a legally sufficient case for rejecting claims 69, 70, and 72-76 under 35 U.S.C. § 103(a) as being unpatentable over Melzak et al., in view of Kleiber et al., and in further view of Ryder et al. (U.S. Patent No. 5,639,599).

INTRODUCTION

The claimed invention relates to methods and kits for isolating a defined and consistent amount of DNA from multiple samples. The importance of being able to isolate a defined and consistent amount of DNA from multiple samples is discussed throughout the specification in the context of performing downstream applications. For example, at page 3, lines 1-21 of the specification, the problems of excess DNA template in amplification reactions are discussed. Traditionally, these problems were addressed by measuring DNA concentrations prior to use in downstream applications. However, measuring DNA concentrations consumes DNA sample and is inaccurate for samples having low concentrations of DNA. (Application as filed, p. 1, lines 30-32.)

The ability to isolate a consistent amount of DNA from multiple samples permits the DNA thus isolated to be used directly in subsequent applications requiring DNA within a particular range without first having to measure the concentration of DNA in order to determine the volume of purified DNA necessary to give an amount of DNA within a suitable range. (*Id.*, p. 5, lines 20-33, p. 9, lines 10-33.) This is particularly important when isolating DNA from relatively scarce sources, such as trace evidence. (*Id.*, p. 1, lines 30-32.) Applicants emphasize that while DNA isolated by the claimed methods is suitable for use in downstream applications without further analysis or processing, the claims do not preclude the possibility of measuring the DNA concentrations. In fact, there are certain applications in which verification of DNA concentration may be required for legal purposes.

During prosecution of the present application, Applicants submitted the declaration of Dr. Rex Bitner, one of the inventors of the claimed invention. In his declaration, Dr. Bitner discusses the importance of being able to isolate a defined and consistent amount of DNA from multiple samples in order to simplify processing, to reduce the time required to process samples, and to increase sample throughput. (*See* Declaration of Rex Bitner under 37 C.F.R. 1.132, attached hereto as Attachment 1, ¶7.) Because DNA isolated from samples using the claimed invention contain a select defined and consistent amount of DNA, the isolated DNA can be used directly in downstream applications requiring an amount of DNA within a particular range. (*Id.*) This represents a departure from prior art methods of isolating DNA, which emphasize maximized DNA yield. (*See id.*, ¶6.)

As Dr. Bitner attests, the claimed methods and kits have received industry praise and addressed a long-felt need in the field. (*See id.*, ¶¶8-16; Exs. B-E.) For instance, Promega

received the prestigious R&D 100 Award for its DNA IQ™ System, a commercial embodiment of the claimed invention, in 2002. (*Id.*, ¶16.) Dr. Bitner also discusses how numerous scientists in the field have acknowledged that Promega's DNA IQ™ System provides a solution to problems they faced with prior art DNA isolation methods, which are very labor-intensive often leading to extensive sample backlogs. (*See id.*, ¶¶5, 7-16; Ex. B (publication indicating DNA IQ™ System isolated uniform amounts of DNA from mock sexual assault samples containing widely varying amounts of DNA that could be used directly in a DNA amplification reaction); Ex. C (correspondence conveying positive experience when using DNA IQ™ System on clinical samples); Ex. D (correspondence reporting DNA IQ™ System decreased total time required to process samples); Ex. E (correspondence acknowledging DNA IQ™ System's ability to greatly increase a laboratory's throughput capabilities, thus permitting the forensic DNA community to provide better service to law enforcement agencies).

ARGUMENT

I. Rejection of claims 44, 45, 50, 53, 66, 67, and 82 under 35 U.S.C. § 102(b) as anticipated by Melzak et al., as evidenced by information available at http://seq.yeastgenome.org/.

Claims 44, 45, 50, 53, 66, 67, and 82 were rejected by the Examiner under 35 U.S.C. § 102(b) as anticipated by Melzak et al., as evidenced by the pUC18 sequence. (*See* Office Action mailed November 18, 2008 ("November 18, 2008 Office Action").) For purposes of this rejection, claims 44, 45, 50, 53, 66, 67, and 82 stand or fall together.

A. Summary of Examiner's Position

The Examiner asserts that all of the limitations of claims 44, 45, 50, 53, 66, 67, and 82 are disclosed by Melzak et al. as evidenced by Figure 3(b). (November 18, 2008 Office Action, pp. 6-8.) The Examiner asserts that Figure 3(b) of Melzak et al. teaches "a DNA titration of silica which shows closed circle data points (i.e. multiple pUC18 samples) forming a saturation curve. Said saturation occurs at and above approximately 4 ug/ml DNA. . . . Melzak et al teach measuring said silica surface area as 5.6 m^2/g, by BET adsorption, reading on claim 44b." (*Id.*, p. 7.) The Examiner further asserts that "in accordance with the open circles in figure 3b of Melzak et al, each of said pUC18 DNA samples are eluted completely (i.e. quantitatively reversibly desorbed), therein each of said closed circle data points represents selecting a defined amount of DNA to be isolated, as set forth in claim 44a," "the DNA samples are introduced to

the silica in excess of the silica binding capacity, reading on claims 44c and 66a and said quantitative reversible desorption of the full capacity of the silica mentioned above reads on claims 44d, 45, and, . . . said eluted DNA may be used in a molecular biology procedure, as set forth in claim 66b," and "the variability of the eluted DNA past the saturation point appears minimal and in the range of claim 82." (*Id.*) The Examiner also asserts that the limitations of claims 50 and 65 are met by the 6 molar perchlorate used by Melzak et al. and that the sequence of pUC18, which allegedly includes short tandem repeats, reads on claims 53 and 67. (*Id.*, p. 8.)

B. Summary of Applicants' Position

The present application was filed in October 2003 and received its first Office Action in February 2006. Applicants have successfully overcome seven references raised by the Examiner over the course of five Office Actions. The disclosure of Melzak et al. does not provide any teaching beyond the many references previously cited and overcome by Applicants. Because prosecution has been extremely protracted due in large part to piecemeal examination, Applicants have elected to appeal the Examiner's rejections to the Board.

1. Melzak et al. reference does not teach or suggest anything beyond previously cited references already overcome by Applicants.

Applicants maintain that Melzak et al. do not anticipate claims 44, 45, 50, 53, 66, 67 and 82 as this reference does not teach or suggest anything beyond prior art references previously cited by the Examiner and overcome by Applicants. For instance, the Examiner previously rejected claims 44-47, 49-52, 54, 55, and 58-74 as anticipated by Smith et al. (WO 98/31840) using essentially the same rational as that advanced in his rejection over Melzak. (*See* July 31, 2007 Office Action, pp. 5-8; November 18, 2008 Office Action, p. 7.) Applicants subsequently amended claims 44-47; 49-52, 54, 55, 58-65, and 68-74 to require the steps of "(a) selecting a defined amount of DNA to be isolated from the samples;" and "(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample." (Oct. 31, 2007 Office Action Response, pp. 2-4.) "[I]n view of applicant's amendments," the Examiner removed the rejection of these claims in view of Smith et al., but maintained his rejection of claims 66 and 67, *i.e.*, the claims Applicants did not amend to require the above-stated limitations. (Nov. 18, 2008 Office Action, p. 4.) Applicants then amended claims 66 and 67 to require the limitations previously incorporated into claims 44-47, 49-52, 54, 55, 58-65, and 68-74 (Feb. 17, 2009 Office Action Response, p. 4), and the Examiner responded

by removing the rejection of claims 66 and 67 as anticipated by Smith et al. (May 1, 2009 Advisory Action, p. 1).

2. Melzak et al. do not teach or suggest the steps of selecting a defined amount of DNA to be isolated from multiple samples, and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA, as required by the rejected claims.

The Examiner's rejection of claims 44, 45, 50, 53, 66, 67 and 82 as anticipated by Melzak et al. is improper because it, like Smith et al., fails to teach or suggest all of the limitations of the rejected claims. The Examiner alleges that "based on the quantitative desorption of pUC18 from silica shown in figure 3B, [Melzak] does indeed isolate consistent amounts of DNA from multiple samples with DNA in excess of the binding capacity as shown in the flat portion of the titration curve." (May 1, 2009 Advisory Action, p. 2.) The Examiner's rejection is erroneous because it fails to consider the claims as a whole, the methods of which include the steps of "(a) selecting a defined amount of DNA to be isolated from the samples;" and "(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample."

The methods disclosed in Melzak et al. were aimed at "determining the dominant forces involved in the binding reaction" of plasmid and chromosomal duplex DNA to silica. (Melzak, abstract.) Figures 3(a) and 3(b) specifically relate to Melzak et al.'s investigation into whether DNA superstructure impacted adsorption characteristics. (*Id.*, p. 639.) Melzak et al. found that washing silica particles which were bound to linearized pUC18 DNA or sonicated salmon sperm DNA silica in a 6 *M* perchlorate solution at 37°C resulted in no appreciable desorption of the linear pUC18 DNA at any surface coverage, *i.e.*, Fig. 3(a), while washing silica particles bound to closed-circular supercoiled pUC18 DNA under the same conditions resulted in near complete removal of supercoiled DNA from the silica surface, *i.e.*, Fig. 3(b). (*Id.*)

Melzak et al. varied the dilution of DNA in the samples merely to illustrate that the amount of DNA in the sample had no impact on whether the binding was reversible. (*Id.*) Thus, the closed and open circles illustrated in Fig. 3(b) do not represent the results of a method of isolating a defined and consistent amount of DNA by selecting a defined amount of DNA to be isolated from multiple samples and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA from the samples, but the results of a method used to determine whether closed-circular supercoiled pUC18 DNA binding to silica in a 6 *M* perchlorate solution at 37°C, unlike linearized DNA, was reversible regardless of the amount of

DNA present in the sample. (*See id.*) Melzak et al. concludes from the results that "[o]pen duplexes (*i.e.*, linear DNA) can establish a continuous line of contact with the silica surface, and thus, bind more strongly than supercoiled DNA under otherwise identical conditions (see Fig. 3)." (*Id.*, p. 643.)

Therefore, Melzak et al. do not teach or suggest the methods of claims 44, 45, 50, 53, 66, 67, and 82, which require isolating a defined and consistent amount of DNA by selecting a defined amount of DNA to be isolated from multiple samples and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA from the samples. In light of the above, the Examiner's rejection of these claims under 35 U.S.C. § 102(b) as anticipated by Melzak et al. is improper.

II. Rejection of claims 44-54, 58, 60-65, 67, 68, and 77-82 under 35 U.S.C. § 103(a) as obvious over Melzak et al., in view of Kleiber et al.

Claims 44-54, 58, 60-65, 67, 68, and 77-82 were rejected in the November 18, 2008 Office Action under 35 U.S.C. § 103(a) as obvious over Melzak et al., in view of Kleiber et al. For the purposes of this rejection, claims 44-54, 58, 60-65, 67, 68, and 77-82 stand or fall together.

A. Summary of Examiner's Position

In support of the rejection of claims 44-54, 58, 60-65, 67, 68, and 77-82 as obvious over Melzak et al., in view of Kleiber et al., the Examiner relies on Melzak et al. for the same reasons as those discussed in Section I(A) above, but concedes that Melzak et al. do not teach magnetic particles (claims 46-49), guanidine thiocyanate (claim 51), genomic DNA (claim 52), further analysis (claim 54), forensic samples (claim 58), heating samples from 60 degrees to 100 degrees (claim 60), sequencing (claim 61), washing with an alcohol and salt (claims 62-63), elution with water (claim 64), Combined DNA Index System Loci (claim 68), DNA amplification (claim 77), sequencing (claim 78), hybridization (claim 79), elution in a discrete volume such that the eluted DNA is from about 0.5 to about 5.0 ng/µl (claims 80-81). (November 18, 2008 Office Action, pp. 9-12.) However, the Examiner asserts that Kleiber et al. teach each of the above limitations including "poreless boro/aluminio/zirconio-silicate magnetic particles useful for DNA isolation" and "separating DNA from said magnetic particles to isolate a defined amount of DNA from ach type of particle." (*Id.*, p. 9.) The Examiner concludes that it would have been *prima facie* obvious for one of skill in the art at the time the claimed invention was made "to apply the

procedure of Melzak et al for discerning the dominant driving forces involved in DNA-silica interactions toward the porous and poreless boro/aluminio/zirconio-silicate magnetic particles according to Kleiber et al." (*Id.*, pp. 10-11.)

B. Summary of Applicants' Position

1. Kleiber et al., like Melzak et al., do not teach or suggest the steps of selecting a defined amount of DNA to be isolated from multiple samples, and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA, as required by the rejected claims.

With regard to the Examiner's rejection of claims 44-54, 60-65, 67, 68, and 77-82 as obvious over Melzak et al., in view of Kleiber et al., Applicants assert that Melzak et al. and Kleiber et al. fail to combine to teach or suggest all of the limitations of these claims. As discussed in Section I(B) above, Applicants assert that Melzak et al. do not teach or suggest the steps of "(a) selecting a defined amount of DNA to be isolated from the samples;" and "(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample." Applicants also assert that "Kleiber does not teach or suggest the problem of isolating defined and consistent amounts of DNA from multiple samples, nor does Kleiber provide the solution of the instantly claimed invention (i.e., selecting a defined amount of DNA to be isolated, [and] choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample . . . ." (Oct. 31, 2007 Office Action Response, pp. 10-11.)

2. Melzak et al. and Kleiber et al. fail to combine to teach or suggest all of the limitations of the rejected claims.

While Kleiber et al. disclose a method of isolating a biological material by bringing a biological sample in contact with magnetic particles under conditions in which the biological material binds to the particle and then separating the biological material from the sample, the methods disclosed by Kleiber et al. are merely aimed at providing and using silica particles to simplify the process of purifying DNA and improve DNA yield. (*See* Kleiber et al., col. 1, ll. 7-15, col. 2, ll. 9-21, Table 1, Examples 3 and 4.) Kleiber et al., like Melzak et al., do not disclose a method of isolating a defined and consistent amount of DNA by selecting a defined amount of DNA to be isolated from multiple samples and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA from the samples. Therefore,

these references fail to combine to teach or suggest all of the limitations of claims 44-54, 60-65, 67, 68, and 77-82.

The non-obviousness of the claimed invention is further supported by secondary consideration evidence, including that the claimed invention received praise from others and satisfied a long-felt need in the industry. *See Vulcan Eng'g Co. v. Fata Aluminum, Inc.*, 278 F.3d 1366, 1373 (Fed. Cir. 2002) (appreciation of the invention by those of ordinary skill in the art is further evidence that the invention would not have been obvious). (*See also* Bitner Decl., ¶¶8-16; Exs. B-E.) As discussed above in the Introduction, Promega received the prestigious R&D 100 Award for its DNA IQ™ System in 2002. (*Id.*, ¶16.) In addition, numerous scientists in the industry have praised Promega's DNA IQ™ System for providing a solution to the problem of being able to isolate a defined and consistent amount of DNA from multiple samples, and doing so in a manner that simplified processing, reduced the amount of sample required and time spent processing, and increased sample throughput. (*See id.*, ¶¶8-16; Exs. B-E.)

Therefore, because Melzak et al. and Kleiber et al. fail to combine to teach or suggest all of the limitations of the rejected claims, and in light of the secondary consideration evidence set forth by Applicants in further support of the non-obviousness of the claimed invention, claims 44-54, 60-65, 67, 68, and 77-82 are not obvious in view of these references. In light of the above, the Examiner's rejection of these claims under 35 U.S.C. § 103 as obvious over Melzak et al., in view of Kleiber et al, is improper.

III. Rejection of claims 69, 70, and 72-76 under 35 U.S.C. § 103(a) as obvious over Melzak et al., in view of Kleiber et al., and in further view of Ryder et al.

Claims 69, 70, and 72-76 are rejected under 35 U.S.C. § 103(a) as obvious over Melzak et al., in view of Kleiber et al., and in further view of Ryder et al. For the purposes of this rejection, claims 69, 70, and 72-76 stand or fall together.

A. Summary of Examiner's Position

The Examiner asserts that Melzak et al. and Kleiber et al. are relied on for the reasons discussed in Section II(A), but concedes that "Melzak et al in view of Kleiber et al do not teach a kit, as set forth in claims 69-70, 72-76." (Nov. 18, 2008 Office Action, p. 12.) The Examiner further asserts that "Ryder et al teach . . . kits containing ferric iron complexing agents for nucleic acid isolation." (*Id.*) The Examiner concludes that "[i]t would have been *prima facie* obvious for one of ordinary skill in the art, at the time the claimed invention was made to include

the reagents used by Melzak et al in view of Kleiber et al with the kit such as described by Ryder et al." (*Id.*)

B. Summary of Applicants' Position

1. Ryder et al. fails to teach or suggest anything beyond Melzak et al. and Kleiber et al.

With regard to the Examiner's rejection of claims 69, 70, and 72-76 as obvious over Melzak et al., in view of Kleiber et al., and in further view of Ryder et al., Applicants assert that these references fail to combine to teach or suggest all of the limitations of the rejected claims. As discussed in Sections I(B) and II(B) above, Applicants assert that Melzak et al. and Kleiber et al. do not, alone or in combination, teach or suggest the steps of "(a) selecting a defined amount of DNA to be isolated from the samples;" and "(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample." Applicants further assert that it is unclear how the interference of ferric ions in downstream applications, or the solutions provided by Ryder et al., has any bearing on the patentability of the claims. (Feb. 17, 2009 Office Action Response, p. 8.)

2. Melzak et al., Kleiber et al., and Ryder et al. fail to combine to teach or suggest all of the limitations of the rejected claims.

Ryder et al. discloses methods and kits for preparing nucleic acids, including isolating DNA or RNA from cells, for amplification. (Ryder et al., col. 1, ll. 38-42.) However, like Melzak et al. and Kleiber et al., Ryder et al. fail to teach or suggest a method of isolating a defined and consistent amount of DNA by selecting a defined amount of DNA to be isolated from multiple samples and choosing a discrete amount of silica-containing solid support necessary to isolate the defined amount of DNA from the samples. Therefore, Melzak et al., Kleiber et al. and Ryder et al. fail to combine to teach or suggest all of the limitations of claims 69, 70 and 72-76. The non-obviousness of claims 69, 70 and 72-76 is further supported by the evidence of secondary considerations discussed above.

In light of the above, the Examiner's rejection of claims 69, 70, and 72-76 under 35 U.S.C. § 103 as obvious over Melzak et al., in view of Kleiber et al, and in further view of Ryder et al. is improper.

CONCLUSION

For the foregoing reasons, claims 44-54, 58, 66-70, and 72-82 should be allowed. Appellants respectfully requests that the Board reverse the rejections and pass the application to allowance.

Respectfully submitted,

Date: 7/17/09

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CLAIMS APPENDIX

1.-43. (Cancelled)

44. (Previously presented) A method for isolating a defined and consistent amount of DNA from multiple samples comprising:

(a) selecting a defined amount of DNA to be isolated from the samples;

(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample;

(c) contacting each sample with the discrete amount of the silica-containing solid support, each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support, under conditions that allow reversible binding of the defined amount of DNA to the solid support; and

(d) separating each sample from the support to isolate a defined and consistent amount of DNA from each sample.

45. (Previously presented) The method of claim 44, further comprising:

(e) separating the DNA of step (d) from the support.

46. (Previously presented) The method of claim 44, wherein the silica-containing solid support comprises silica magnetic particles.

47. (Previously presented) The method of claim 46, wherein the silica magnetic particles are porous.

48. (Previously presented) The method of claim 46, wherein the silica magnetic particles are nonporous.

49. (Previously presented) The method of claim 46, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.

50. (Previously presented) The method of claim 44, wherein the conditions comprise the presence of a chaotropic salt.

51 (Previously presented) The method of claim 50, wherein the chaotropic salt comprises guanidine thiocyanate.

52. (Previously presented) The method of claim 44, wherein the DNA is genomic DNA.

53. (Previously presented) The method of claim 44, wherein the DNA is plasmid DNA.

54. (Previously presented) The method of claim 44, further comprising analyzing the defined amount of DNA of step (d).

55. (Withdrawn) The method of claim 44 wherein the sample comprises a solid support.

56. (Withdrawn) The method of claim 55 wherein the solid support of the sample is paper.

57. (Withdrawn) The method of claim 55, wherein the solid support of the sample is a swab.

58. (Previously presented) The method of claim 44 wherein the sample is a forensic sample.

59. (Withdrawn) The method of claim 55, wherein the sample is contacted with a chaotropic salt.

60. (Previously presented) The method of claim 59, wherein the contacted sample is heated to a temperature of from about 60° to about 100°C.

61. (Previously presented) The method of claim 44, further comprising determining at least a portion of the sequence of the isolated DNA.

62. (Previously presented) The method of claim 45, further comprising washing the solid support prior to step (e).

63. (Previously presented) The method of claim 62, wherein the solid support is washed with a solution comprising an alcohol and a salt.

64. (Previously presented) The method of claim 45, wherein the DNA of step (e) is separated by eluting with water.

65. (Previously presented) The method of claim 50, wherein the concentration of chaotropic salt is between about 0.1 M and 7 M.

66. (Previously presented) A method of isolating DNA from multiple samples for use in a molecular biological procedure comprising:

(a) selecting a defined amount of DNA to be isolated from the samples;

(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample;

(c) contacting each sample with a discrete amount of a silica-containing solid support, each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support, under conditions that allow reversible binding of the defined amount of DNA to the solid support; and

(d) eluting bound DNA of step (c) to isolate a defined and consistent amount of DNA from each sample, wherein the eluted DNA is suitable for use in the molecular biological procedure.

67. (Previously presented) The method of claim 66, wherein the molecular biological procedure includes analysis of at least one DNA sequence comprising at least one short tandem repeat sequence.

68. (Previously presented) The method of claim 67, wherein the at least one short tandem repeat sequence comprises the Combined DNA Index System loci.

69. (Previously presented) A kit for isolating a defined and consistent amount of a DNA from multiple samples according to claim 44, the kit comprising:

silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles.

70. (Previously presented) The kit of claim 69 wherein the sample comprises blood.

71. (Withdrawn) The kit of claim 69, wherein the sample comprises a solid support.

72. (Previously presented) The kit of claim 69, further comprising a chaotropic salt.

73. (Previously presented) The kit of claim 69, wherein the silica magnetic particles are provided in a solution comprising the chaotropic salt.

74. (Previously presented) The kit of claim 69 further comprising a wash solution.

75. (Previously presented) The kit of claim 69, wherein the silica magnetic particles are siliceous oxide-coated magnetic particles.

76. (Previously presented) A kit for isolating a defined and consistent amount of DNA from multiple samples according to claim 66, the kit comprising, silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles.

77. (Previously presented) The method of claim 66, wherein the procedure is a DNA amplification reaction.

78. (Previously presented) The method of claim 66, wherein the procedure is a DNA sequencing reaction.

79. (Previously presented) The method of claim 66, wherein the procedure is a DNA nucleic acid hybridization.

80. (Previously presented) The method of claim 66, wherein the DNA of step (d) is eluted in a discrete volume to provide a solution having a defined DNA concentration suitable for use in the procedure without separate quantification.

81. (Previously presented) The method of claim 80, wherein the DNA concentration is from about 0.5 ng/μl to about 5.0 ng/μl and the procedure is a DNA amplification reaction.

82. (Previously presented) The method of claim 44, wherein the defined and consistent amount of DNA isolated is within 60% to 229% of the mean amount of DNA isolated from the samples.

EVIDENCE APPENDIX

1. Attachment 1: Declaration of Rex Bitner under 37 C.F.R. 1.132, including Exhibits A-E attached thereto.

RELATED PROCEEDINGS APPENDIX

None.

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant(s):	Tereba et al.	**Docket No.:**	016026-9043-US01
Serial No.:	10/694,475	**Group Art Unit:**	1639
Filing Date:	October 27, 2003	**Examiner:**	Christopher M. Gross
Title:	SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA		

DECLARATION OF REX BITNER UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Rex Bitner, do hereby declare and state the following:

1. I have served as the Technology Manager of the Genetic Analysis R&D group at Promega Corporation, Madison, WI, since 2003. From 1997-2003, I worked as a Senior Scientist and Senior Project Manager of R&D at Promega Corporation. I hold a B.S. in Biology from The University of Washington, Seattle, WA and a Ph.D. in Genetics from The University of California, Davis, CA. A copy of my curriculum vitae is attached as Exhibit A.

2. I am a joint inventor of at least some of the claimed subject matter of the above-identified patent application. I make this declaration in support of prosecution of the present application before the U.S. Patent and Trademark Office.

3. I have read and understand the invention as disclosed in the present application, including the invention described by the presently pending claims. I have also reviewed the July 31, 2007 Office Action. I understand that each of claims 44-52, 54, 55, and 58-68 is rejected as being unpatentable (i.e., obvious) over Kleiber et al. (WO 96/41811), Huber et al. (1993 Nuc. Acids Res. 21:1061-1066), and Vogelstein et al. (1979 PNAS 76:615-619).

4. Claims 44-52, 54, 55, and 58-68 are directed to methods of isolating a defined and consistent amount of DNA from multiple samples by choosing the amount of DNA to be

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isolated, choosing an amount of a silica containing solid support needed to isolate the defined amount of DNA, such that the amount of DNA in the samples is greater than the binding capacity of the solid support, and contacting each sample with the solid support under conditions that allow isolation of the defined and consistent amount of DNA.

5. DNA IQ™ System is the term that Promega Corporation, the assignee of the instant application, uses with its customers when referring to methods for isolating a defined and consistent amount of DNA from multiple samples, as described throughout the application and as summarized in paragraph 4, above.

6. The concept underlying the methods of the invention, which focus on isolating just a portion of DNA that may be present in a sample, represents a complete departure from prior art methods such as those described in Kleiber et al. and Vogelstein et al., which focus on maximizing DNA yield. For example, Kleiber et al. discusses the relatively high yields obtained by their methods. (Please see, for example, page 12 of Kleiber et al.). Similarly, Vogelstein et al. emphasizes that binding DNA to glass from dissolved agarose "is rapid, convenient, and nearly quantitative." (Vogelstein et al., p. 618, first column, last line to second column, first line, emphasis added). Huber et al., rather than isolating a defined and consistent amount of DNA, describes using high performance liquid chromatography (HPLC) to fractionate relatively small (i.e., <500 bp) DNA fragments such as restriction fragments and PCR products according to size.

7. The ability to isolate a defined and consistent amount of DNA from multiple samples using the methods of the invention simplifies processing, reduces the amount of time needed to process samples, and increases sample throughput. Because DNA samples prepared using the method of the invention contain a select defined and consistent amount of DNA, the isolated DNA can be used directly in downstream applications requiring an amount of DNA within a particular range.

8. The importance of increased sample throughput in isolating DNA cannot be overstated. For example, because prior art methods of isolating DNA from samples were very labor-intensive and cost about $1000 per test, a backlog of several hundred thousand samples from rape victims awaited processing in 1999 when the patent application for the present invention

was submitted. Thus, samples containing evidence that may have been useful in identifying sex offenders go unprocessed.

9. Attached as Exhibit B is an article entitled "Robotic Extraction of Mock Sexual Assault Samples using Biomek® 2000 and the DNA-IQ™ System", authored by Susan Greenspoon and Jeff Ban of the Virginia Division of Forensic Science, which appeared in the February 2000 issue of Profiles in DNA.

10. Greenspoon and Ban describe how the DNA IQ™ System, used in conjunction with the Biomek® 2000 robotics system, was able to isolate uniform amounts of DNA from mock sexual assault samples containing dilutions of semen of from 1:10 to 1:200 on a ½, ¼, or 1/8 portion of a swab (See p. 4, Mock Sexual Assault Samples). The DNA thus isolated from samples having widely varying DNA content was used directly in PowerPlex® 1.1 System, a DNA amplification reaction used in genetic identity testing, and produced uniform results.

11. Other experts in the field have recognized the importance of the instantly claimed methods isolating DNA for use in molecular biological methods such as amplification for genetic identity testing.

12. Dr. Weimin Sun, Scientific Director in the Molecular Genetics Department of Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, evaluated the DNA IQ™ System for use in clinical samples and found that the methods yielded consistent quantities of DNA despite significant variations in the starting material, which afforded satisfactory performance in downstream applications. (See correspondence form Dr. Sun to Mr. David Phelps, Exhibit C).

13. Kim Gorman, then President of Paternity Testing Corporation, Columbia, MO, reported that the DNA IQ™ System was used to extract DNA from buccal swabs in a 96 well format. (See correspondence from Ms. Gorman to Mr. Phelps, Exhibit D). Ms. Gorman noted that buccal swabs vary greatly in DNA content, and also noted that DNA multiplexes (amplification of multiple DNA loci in a single reaction) are concentration sensitive. Despite these challenges, Ms. Gorman further noted that there was no need to quantify the DNA prepared using the DNA IQ extraction method prior to use in a multiplex reaction. Ms. Gorman reported that, using the DNA IQ™ System, the total time required to process 96 samples was reduced from 6 or 7 hours

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using their traditional extraction method to about 3 hours, and hands on time by analysts was reduced from more than 4 hours to less than 30 minutes.

14. Jeffrey Ban, Section Chief of Forensic Biology at the Virginia Department of Criminal Justice Services, Division of Forensic Science, reported that, using the DNA IQ™ System in conjunction with Beckman Coulter Biomek® 2000 Workstation or other similar instrumentation, could greatly increase a laboratory's throughput capabilities, thus permitting the Forensic DNA community to provide better service to law enforcement agencies (See correspondence from Mr. Ban to Mr. Phelps, Exhibit E).

15. In addition, Promega has been contacted by numerous law enforcement agencies that ultimately used the DNA IQ™ System to analyze forensic samples from crime scenes. For example, the Royal Canadian Mounted Police requested assistance in processing samples collected from a hog farm in British Columbia, where the partial remains of at least 26 murder victims were found. The DNA IQ™ System was also used to isolate DNA for genetic identity testing of victims at Ground Zero and human remains found in mass graves in Bosnia.

16. In 2002, Promega received an R&D 100 Award for its DNA IQ™ System. Through the R&D 100 Awards program, sponsored by R&D Magazine, organizations receiving the awards are recognized for the most technologically significant products introduced into the marketplace.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: October 30, 2007

Rex Bitner

Rex Bitner

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QUALIFICATIONS SUMMARY

Over twenty years of industrial experience in biotechnology research and product development at Promega (Madison, WI), Amersham/Pharmacia Biotech (now GE Healthcare), (Milwaukee, WI), and 3M (St. Paul, MN). Four years of postdoctoral research in molecular biology at the University of Colorado, Boulder and the University of California, Davis. Broad, in-depth knowledge of molecular biology, solid phase purification of biological materials, and all aspects of product development in an ISO9001: 2000 environment. Experienced in technology management, project leadership and management, supervision of a BL3 laboratory, and the development, launch and care for biotechnology products. Extensive experience in the creation, development and evaluation of intellectual property, including USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, as well as participation in foreign patent opposition proceedings, both as proprietor and as an opposition party, and as an opposition member in the appeal of an invalidated patent.

PROFESSIONAL HISTORY

1997 - present **PROMEGA CORPORATION, Madison, Wisconsin**

Technology Manager, Genetic Analysis, R&D (2003 - present)

Technology Manager, Genetic Analysis: management of a laboratory group, development of separation technologies, intellectual property, and purification products for the biotechnology marketplace and clinical laboratory market, with particular emphasis on automation of nucleic acid purification products for use in genomics, high throughput pharmaceutical drug screening, and clinical diagnostics. Intellectual property management has included USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, phone interviews with USPTO and foreign patent examiners, and participation in European patent opposition proceedings, both as proprietor and as an opposition party, including as an opposition member in the appeal of an invalidated patent. The efficient integration of foreign patent filing strategies and licensing of intellectual property within Promega's Genetic Analysis business strategy has been a central responsibility.

Senior Scientist, Senior Project Manager, R&D (1997 - 2003)

Development of pH dependent ion exchange purification systems using both column and paramagnetic particle purification methods in robotic workstations, with additional emphasis on automated cell concentration and magnetic clearing of cellular lysates in 96-well walkaway automated DNA purification (particularly using Beckman BioMek® FX and Tecan Genesis® robotic platforms). Products developed for genomic DNA purification from human whole blood and tissues (including R&D Magazine's R&D 100 award winning DNA-IQ™(for 2002), plant materials, and DNA purification from food ingredients for use in the quantitative detection of genetically modified organisms (GMO) in food. Additional experience with DNA sequencing automation, RNA purification, and PCR cleanup, particularly using Beckman, Tecan and Thermo-Electron Labsystems robotic platforms.

Project leader for Promega products, in an ISO9001: 2000 environment, including:

Wizard® Genomic, 10ml blood	A1620	Wizard® Magnetic DNA Purification for Food	FF3751
Wizard® Magnetic 96 DNA Plant	FF3761	MagneSil® Blood Genomic, Max Yield	MD1360
MagneSil® ONE, Fixed Yield	MD1370	MagneSil® KF, Genomic System	MD1460
PureYield™ RNA Midi-Prep System	Z3741	PureYield™ Plasmid Midi-Prep System	A2495

PROFESSIONAL HISTORY, continued

1995 - 1997 **AMERSHAM PHARMACIA BIOTECH INC., Milwaukee, Wisconsin.**

Senior Research Scientist (1995 - 1997)

Senior Research Scientist and Project Leader responsible for nucleic acid purification products for the biotechnology laboratory: development of novel separations matrices and processes, formulation and execution of project plans, maintenance of timelines and scheduling, and supervision of personnel on several project teams. Research and development of new products for the molecular biology marketplace, particularly in the areas of proprietary purification products, anion exchange chromatography, and solid phase extraction and immobilization of nucleic acids. Responsibilities included the management of personnel, timelines and ISO 9001 documentation of product development.

1982 - 1994 **3M COMPANY, St. Paul, Minnesota.**

Research Specialist (1984 - 1994)

Identifying, planning and pursuing molecular biology programs of interest to 3M businesses. Research programs involved diverse product objectives: Development of 3M's Rapid Attest™ biological/sterilization monitor product, GMP purification of bovine phosphophoryn proteins (for bone repair), genetic manipulation of bacteria to produce specialty chemicals (*meta*-hydroxyphenylacetylene, and aromatic compounds useful in laser dyes), cDNA cloning of mammalian genes for drug discovery screening, surface immobilization of nucleic acids onto ceramic oxide/3M Empore™ (PTFE) membranes (for use in DNA blotting, hybridization and sequencing), solid phase extraction of DNA for automated sequencing, solid phase extraction of DNA from human blood plasma for use in PCR, protein immobilization on azlactone functionalized porous beads (including 3M Emphaze™ beads and network beads), and cloning of stress protein genes from bacteria associated with periodontal disease. Development and implementation of DNA purification technologies, using a variety of ceramic matrices for solid phase extractions. Over ten years experience supervising a biosafety level 3 containment laboratory.

Other responsibilities included evaluation of both internal and external research proposals and intellectual property. Additional responsibilities as Institutional Biosafety Officer, OSHA blood-borne pathogen safety officer, and as scientific advisor in the development of and compliance with Minnesota State (Environmental Quality Board) regulations governing recombinant organisms.

Senior Biologist (1982 -1984)

Responsibilities included setting up and staffing a recombinant DNA laboratory, evaluation of outside business proposals and intellectual property issues, and initiation of new research programs in molecular biology: gene expression in *Bacillus subtilis*, and R&D of 3M's Rapid Attest™. Additional responsibilities: initiation of university research contracts and management of laboratory personnel.

1978 - 1982 **UNIVERSITY OF COLORADO, Boulder, Colorado.**

Postdoctoral research: Dr. Peter L. Kuempel, Dept. of Molecular, Cellular, and Developmental Biology: Termination of chromosome replication in *E. coli*.

PROFESSIONAL HISTORY, continued

1974 - 1978 **UNIVERSITY OF CALIFORNIA, Davis, California.**

Postdoctoral research: Dr. Gordon G. Edlin, Dept. of Genetics (1978).
Instructor: Department of Genetics (1977).

1974 **UNIVERSITY OF WASHINGTON, Seattle, Washington**
Post-graduate Research Assistant: Dr. Jonathan A. Gallant, Dept. of Genetics

EDUCATION

Ph.D. in Genetics, 1978
University of California, Davis, California

B.S. in Biology, *cum laude*, 1974
University of Washington, Seattle, Washington

PATENTS

Rex M. Bitner, Mark A. Denhart, Don Smith. US Utility Application: WO2007/103485. Small RNA Purification.

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Craig E. Smith, Diana L. Holmes, Daniel J. Simpson, Jehoshua Katzenhendler, Rex M. Bitner, Josephine C. Grosch. US 6,376,194, US 6,270,970, EP1179056, AU4841500 and WO0070041. Mixed Bed Solid Phase and Its Use in the Isolation of Nucleic Acids.

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Rex M. Bitner, Chan-Wha Kim, and Michael G. Williams. EP647232B1, WO9400464. 3M, St. Paul, MN. Deproteinization with azlactone-functional supports.

Rex M. Bitner and Eric F. Funkenbusch. EP0391608. Applicant: Minnesota Mining and Manufacturing Company, St. Paul, MN. Metal oxide supports for nucleic acids.

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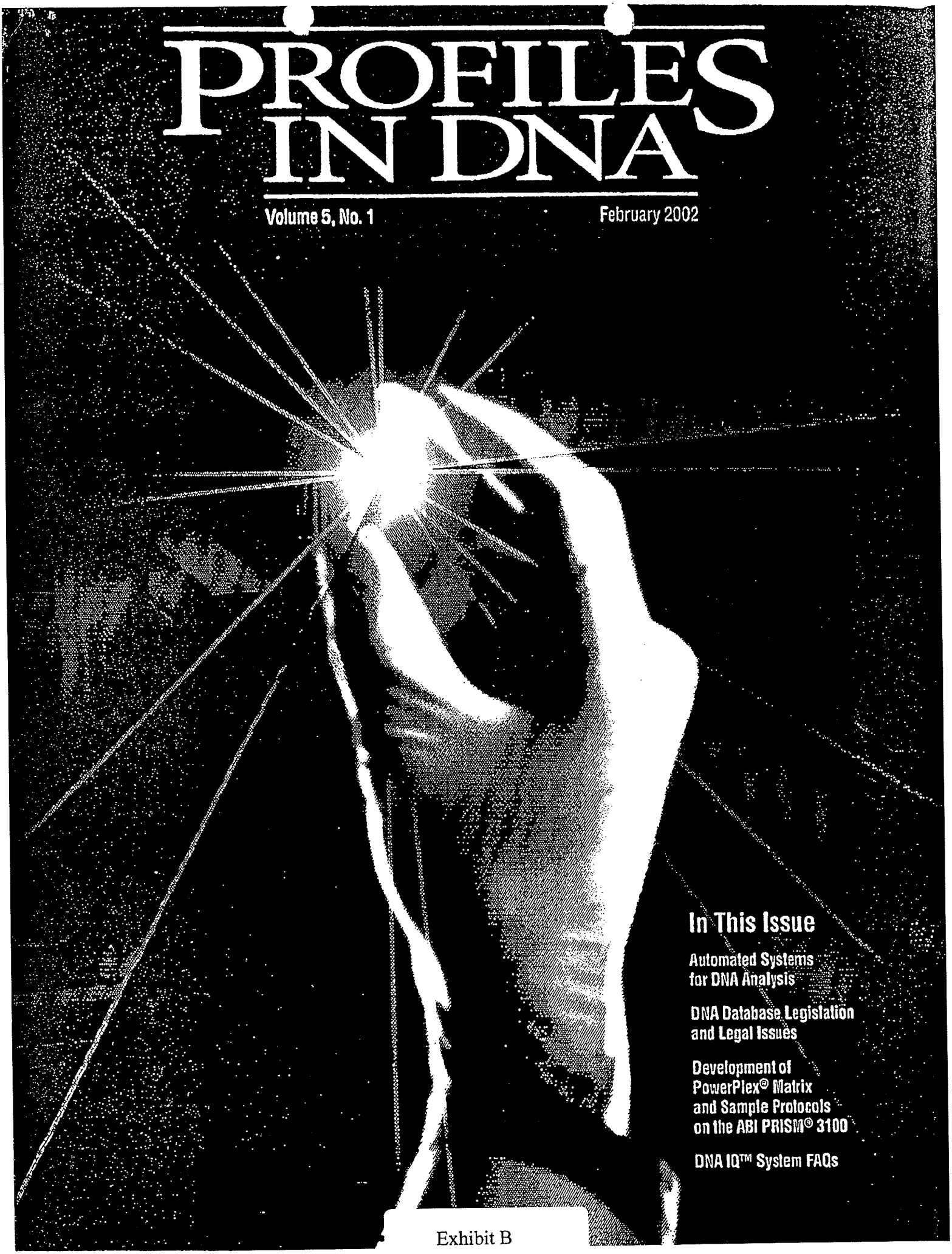
PROFILES IN DNA

Volume 5, No. 1 February 2002



In This Issue

Exhibit B

Robotic Extraction of Mock Sexual Assault Samples Using the Biomek® 2000 and the DNA IQ™ System

By Susan Greenspoon and Jeff Ban
Virginia Division of Forensic Science, Richmond, Virginia

The Biomek® 2000 used in conjunction with the DNA IQ™ System has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

INTRODUCTION

Forensic scientists are routinely faced with the challenge of isolating DNA from a large array of tissue and cell types. The variety of substrates upon which cellular material has been deposited, some of which may contain inhibitors of PCR, can make the process more difficult (1). Therefore, any robotic system applied to the extraction of forensic casework samples must be robust enough to address these variations. The Biomek® 2000 used in conjunction with the DNA IQ™ System(a) has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

The DNA IQ™ System uses silica-coated magnetic beads to separate DNA from cellular debris. Cells are lysed in a powerful lysis buffer, and the lysate is mixed with the magnetic beads. The beads saturate at approximately 100ng of bound DNA, and the excess DNA is removed by pipetting. Once bound to the magnetic resin, the DNA is pipetted and vigorously shaken several times in wash buffer, then eluted using heat. The Biomek® 2000 is equipped with a magnetic plate, a shaking platform and a thermal exchange unit to perform these necessary steps.

CONTAMINATION STUDIES

A number of exploratory and validation studies have been performed on the Biomek® 2000/ DNA IQ™ System to evaluate the viability of this automated system for use with forensic samples. Fundamental questions needed to be answered before moving ahead with extensive validation work. First, does the robotic, open-plate format cause contamination? To answer this question, two sample formats were used repeatedly, and extracted samples were analyzed to test

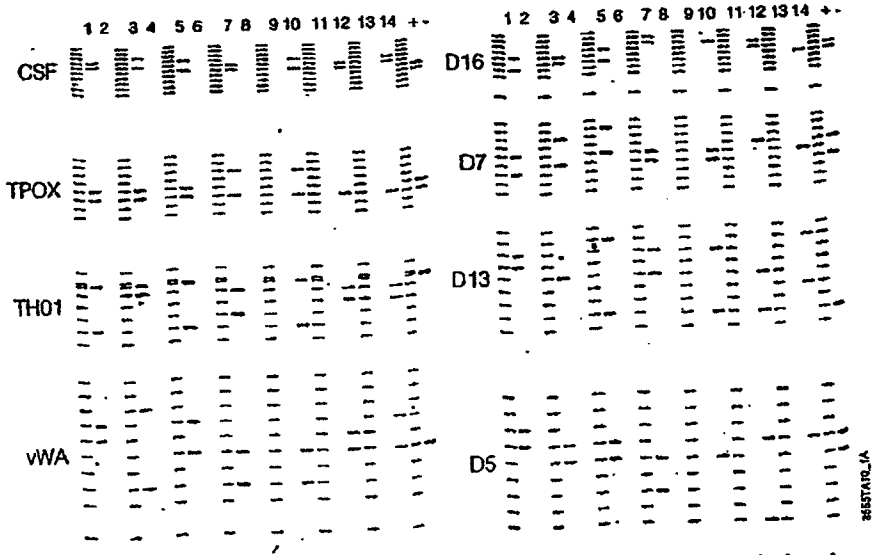


Figure 1. Amplified DNA samples using the PowerPlex® 1.1 System from the 88-sample checkerboard contamination study. Numbers 1–14 indicate sample numbers, "+" indicates positive control (K652), and "–" indicates negative control. Amplified DNA was analyzed by electrophoresis using a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

for contamination. The first is the zebra-stripe format test: alternating columns of samples containing an abundant source of DNA with columns containing reagent blanks (8 sample wells per column). Therefore, a column of samples containing abundant DNA was processed adjacent to a column of reagent blanks in a striped pattern on the plate. The samples containing DNA were bloodstains cut into 5mm² squares. DNA was eluted from the magnetic beads into 100µl of sterile water, then quantified, amplified, and typed using the PowerPlex® 1.1 System[b,c] (2). The first two trials of this test detected some contamination. The software method used was modified to accommodate sample loading into a 96 deep-well plate in place of the more shallow Greiner plate and to remove an initial shaking step. A subsequent zebra stripe experiment showed no contamination with the 40 samples that were isolated.

The second contamination test is a checkerboard sample format: samples containing abundant DNA were alternated with reagent blanks in a checkerboard pattern across a 96 deep-well plate (Figure 1). All 128 samples (88 sample and 40 sample methods) tested negative for any detectable contamination.

MOCK SEXUAL ASSAULT SAMPLES

Sexual assault cases frequently constitute the majority of DNA cases received by a forensic laboratory. Presently, no robotic system is available that can separate sperm from non-sperm cells and thus perform a differential extraction (3) from start to finish. However, the first step of separating fractions can be performed manually. Subsequently, the DNA from E-cell (epithelial or non-sperm cell) lysates and sperm pellets can be extracted robotically, saving analysts a substantial amount of time. Any robotic extraction of sexual assault samples must at least be able to generate sample DNA, equivalent in both quality and yield to that generated by manual DNA extraction methods. Therefore, a thorough examination of the Biomek® 2000/DNA IQ™ System's ability to isolate DNA from sexual assault samples needed to be performed. The first step was to ascertain whether sperm cells could be successfully lysed and the DNA purified by the robotic system. Mock sexual assault samples were prepared using previously donated vaginal swabs and semen from a known donor, which was deposited onto sterile cotton swabs in 1:2 and 1:4 dilutions. The E-cells were lysed manually, the sperm cells pelleted, and a portion of the lysates and the entire sperm pellets were loaded onto the Biomek® 2000 for DNA extraction. DNA was eluted off the magnetic beads into 100µl of sterile water. High-quality DNA was obtained and typed accurately using the PowerPlex® 1.1 System (data not shown).

Once it was demonstrated that the Biomek® 2000/DNA IQ™ System could successfully complete the differential extraction process with the E-cell lysates and intact sperm, the next question addressed was whether this automated system could produce DNA of comparable quality and yield to that produced by manual extraction. A comparative study was designed to measure the performance of the Biomek® 2000/DNA IQ™ System with respect to manual extraction of similar if not identical samples. Samples were prepared in the following manner:

1. Sets of vaginal swabs from 5 different donors were selected.
2. Duplicate mock sexual assault swabs were prepared using semen from a single donor at the following dilutions: 1:10, 1:100, 1:1,000 and 1:10,000 for three sets, 1:10, 1:100, 1:200 and 1:400 for one set and 1:100, 1:200, 1:400 and 1:800 for the last set.
3. Once dried, the swabs were cut into 1/2, 1/4 and 1/8 portions.
4. The E-cells were lysed and the sperm cells pelleted and washed.
5. The samples were split evenly with one half going to an analyst to complete the extraction manually and the other loaded onto the Biomek® 2000 for a robotic DNA extraction.

Yields and quality of the DNA from the sperm fractions processed by the Biomek® 2000/DNA IQ™ System were comparable and frequently superior to those obtained by manual extraction (Figure 2). In less experienced hands, the Biomek® 2000/DNA IQ™ System clearly outperformed the manual extraction (data not shown). Results obtained by experienced users were equivalent to those achieved with the robot. Therefore, the Biomek® 2000/DNA IQ™ System is not only capable of outperforming its human counterpart, but it also delivers a more consistent product.

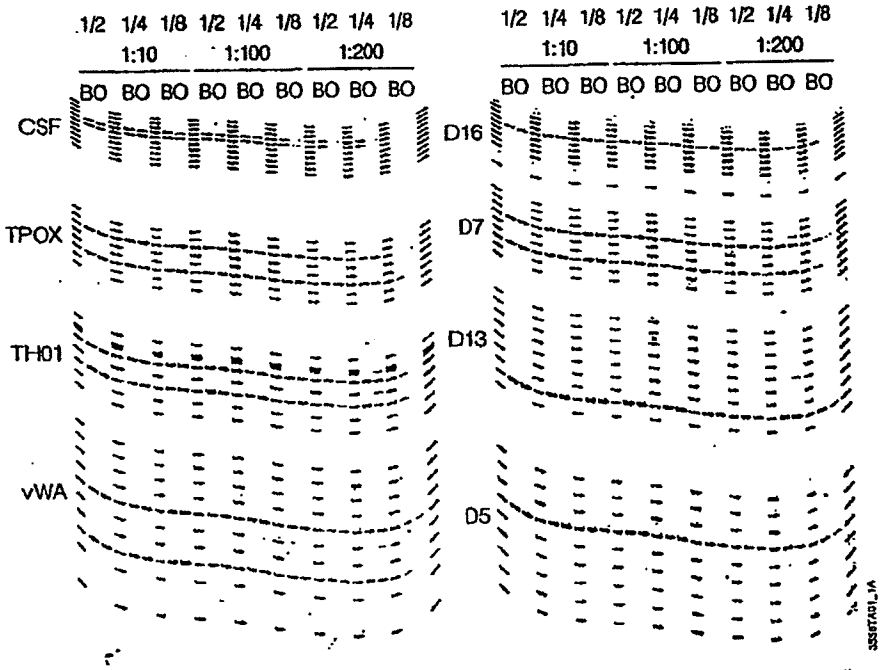


Figure 2. PowerPlex® 1.1 mock sexual assault comparison study. Duplicate samples were extracted manually (indicated by an "O" above the lane, for organic) or robotically (indicated by a "B" above the lane, for Biomek® 2000). Semen dilutions (1:10, 1:100 and 1:200) for each set of swabs are indicated above the corresponding six sample lanes, which contain DNA extracted from the indicated swab portion (1/2, 1/4 or 1/8). Amplified DNA was separated by electrophoresis in a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

Exhibit B

The maximum sample volume for use with the 96 deep-well plate is limited to 100µl. Sperm cells are typically in a pellet of 50µl and therefore unaffected by the volume limit. Since the E-cell lysate is usually in a volume of 500µl, it was important that the yields from 100µl of E-cell lysate from the 1/2, 1/4 and 1/8 swab portions be sufficient for all DNA typing needs. Total yields of E-cell DNA extracted on the robot were calculated (Figure 3), and sufficient E-cell DNA could be obtained using robotic extraction methods. In fact, 1/8 swab portions provided more than enough DNA for an E-cell DNA profile.

The amount of time saved by using the Biomek® 2000/DNA IQ™ System to extract the forensic samples can be substantial. The time it takes to complete the organic extraction manually for a single sample is 5 hours and 5 minutes (after E-cells have been lysed, and sperm cells pelleted and washed). Of course, additional samples will lengthen the amount of time proportionately. In comparison, the robot takes 1 hour and 15 minutes to extract the DNA from 40 samples and 1 hour and 50 minutes to extract 88 samples. Therefore, the absolute minimum amount of time saved is 3 hours and 50 minutes or half of a day.

EXTRACTION OF OTHER CELL AND TISSUE TYPES

Since a variety of cells and tissue types are encountered in routine forensic casework, the Biomek® 2000/DNA IQ™ System was evaluated to determine its capability to isolate DNA from a variety of sources. Dried bloodstains, E-cell lysates, intact sperm cells, muscle, heart, brain, liver and buccal swabs were extracted using the Biomek® 2000/DNA IQ™ System and successfully typed using the PowerPlex® 1.1 System (data not shown).

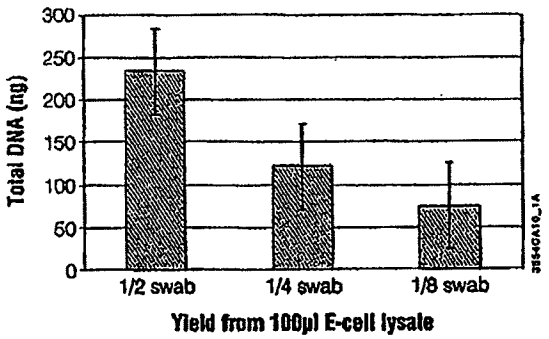


Figure 3. Bar graph depicting the total yield of E-cell DNA generated from extraction on the Biomek® 2000 robot using 100µl of lysate. Yields were determined by measuring DNA concentration using the QuantiBlot® kit.

CONCLUSION

A completely automated system for extraction of sexual assault samples is currently not available. However, once the E-cells have been separated from the sperm cells, robotic DNA extraction using the Biomek® 2000/DNA IQ™ System can be accomplished. Moreover, when sperm DNA is limited, the Biomek® 2000/DNA IQ™ System generates DNA of similar, and sometimes better, quality and yield than that obtained by manual extraction of a duplicate sample. Because of the adaptability of the Biomek® 2000/ DNA IQ™ System, this instrument has the potential to handle future applications of emerging cell separation technologies. It may be possible on a robotic platform, to separate sperm cells from non-sperm cells with the use of an anti-sperm antibody conjugated to a magnetic bead. One can envision a completely automated system where both cell separation and DNA extraction are performed on the same robot. The Biomek® 2000/DNA IQ™ System may be uniquely poised to proceed with that application when the technology becomes available.

The time saved when compared with manual extraction, as well as the ability to extract a variety of tissue and cell types, makes the Biomek® 2000/DNA IQ™ System attractive for casework applications. Further validation work on the Biomek® 2000/DNA IQ™ System must be performed in order to complete our evaluation and validation prior to its application for forensic casework. Although no contamination of the samples was detected after modifying the method and changing the format, special circumstances might require the use of manual extraction methods, for example, when an evidentiary sample may be completely consumed due to limited available material.

ACKNOWLEDGMENTS

We would like to acknowledge the participation of other members of the Virginia Division of Forensic Science: Beth Ballard, Missy Baisden, Brian Covington, Shelley Smith and Colleen Young. We would also like to thank Allan Tereba and Dan Kephart at Promega Corporation for all their hard work at making the technology and this study possible.

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(a,b,c)Refer to the patent and disclaimer statements on page 2.

Exhibit B

33608 Ortega Highway
San Juan Capistrano, California 92690
949.728.4000

March 4, 2002

David Phelps
Promega
Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

Dear Mr. Phelps,

We have evaluated DNA IQ system for isolation of DNA from clinical specimens (whole blood and buccal swabs). The IQ system showed satisfactory performance on both types of samples. It is highly tolerant to the variability of sample input quantity. It also demonstrated high consistency in the quantity of product DNA. This feature is extremely desirable to us as buccal swab samples can have significant variations in starting material affected by collection procedure and skills. In addition, DNA samples isolated using the DNA IQ system have shown satisfactory performance in the downstream applications and have excellent stability as well.

If there is any question in regards to our experience with the DNA IQ system, please do not hesitate to contact me.

Sincerely,

Weimin Sun, Ph.D., ABMG.
Scientific Director
Molecular Genetics Department
Quest Diagnostics Nichols Institute
33608 Ortega Highway
San Juan Capistrano, CA 92690
(949) 728-4498 (voice)
(949) 728-4874 (fax)

Paternity Testing Corporation

Fast, Confidential DNA *Analysis*

February 25, 2002

David Phelps
Promega, Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

VIA FACSIMILE: (608) 273-6455

Dear Mr. Phelps:

Lisa Lane requested that I send you a brief letter about our experience using the DNA IQ extraction method.

PTC has been extremely interested in automating DNA extractions from buccal swabs. Because of the nature of buccal swabs it has historically been extremely difficult if not impossible to work with them in a 96 well automated format. Swabs absorbed liquid and had to be spun through a basket in order to recover the lysis buffer. Swabs had to be removed by hand one at a time. There were many transfers in and out of centrifuges. It was a very labor intensive process to carry out the extractions. It was possible to automate the extractions, but that required large volumes and could not be carried out in a 96 well format.

Our goal was to be able to put a buccal swab into a 96 well tray and never have to touch it again. DNA IQ allows for extractions to be carried out in the 96 well format from the time the specimens are placed into a 96 well deep well tray until the DNA is transferred to the amplification tray.

DNA IQ also has the advantage of eliminating the need to quantify the DNA. Buccal swabs vary greatly in DNA content. Since the DNA multiplexes are somewhat concentration sensitive, it was necessary to get a rough quantification of the amount of DNA present then diluting to appropriate volumes. There is no need to quantify the DNA using the DNA IQ extraction method. The concentration of DNA is consistent from well to well. By using 1µl of the isolated DNA we are able to get consistent results from virtually all specimens.

3501 Berrywood Dr. • Columbia, MO 65201
Toll Free 1-888-837-8323 • Phone: (573) 442-9948 • Fax: (573) 442-9870
Email: ptc@ptclabs.com • Website: www.ptclabs.com

DNA IQ is also much faster than most extraction methods. It takes approximately 3 hours from the time the swabs are placed into the tray until the DNA is pipetted into the amplification tray. There is less than 30 minutes of analysts time involved in the entire process. Using our traditional extraction method it takes 6 or 7 hours to prepare 96 samples for amplification and more than 4 hours of actual hands on time by the analysts.

Our lab has nearly completed the final stages of testing and validation of DNA IQ. We plan to be online using DNA IQ in March.

Yours truly,

Kim Gorman

Kim Gorman
President

COMMONWEALTH of VIRGINIA

DEPARTMENT OF CRIMINAL JUSTICE SERVICES

DIVISION OF FORENSIC SCIENCE
CENTRAL LABORATORY
A Nationally Accredited Laboratory

P.O. BOX 999
RICHMOND, VIRGINIA 23218
(804) 786-4707

February 25, 2002

David Phelps
Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399

Dear Mr. Phelps,

In order to aid the DNA examiner in the extraction of casework samples, the Virginia Division of Forensic Science, Forensic Biology Section, for the past 6 months has worked with scientists and engineers from the Promega Corporation and Beckman Coulter to evaluate, modify and validate the Beckman Coulter Biomek® 2000 Workstation in conjunction with the Promega DNA IQ™ Isolation System. Currently each DNA examiner spends between 6 and 8 hours purifying the DNA from an average sexual assault case consisting of 3 to 5 evidence samples. Because of the adaptability of the Promega DNA IQ™ Isolation System the DNA purification can be automated permitting the user to walk away once the samples are setup in the robot.

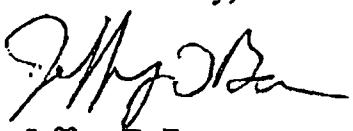
In the time it would takes 4 DNA case examiners (i.e. between 24 and 32 person hours) to manually purify the DNA from evidence samples from 3 to 5 cases, using an organic extraction procedure, the robot can accomplish in under 2 hours due to the speed of the Promega DNA IQ™ purification process paired with the Beckman Coulter Biomek® 2000 Workstation. Therefore, with as little as 5 to10 minutes of a technician's time to setup the instrument, the Virginia Division of Forensic Science, Forensic Biology Section can improve the turnaround time of cases with minimum impact on the casework examiner.

Utilizing the chemistry of the Promega DNA IQ™ Isolation System in combination with the Beckman Coulter Biomek® 2000 Workstation or similar instrumentation can greatly increase a laboratory's throughput capabilities for analyzing forensic casework samples. Thus in turn

Exhibit E

permitting the Forensic DNA community to provide a better service to the law enforcement agencies throughout the United States to help solve crimes against persons and property.

Yours sincerely,



Jeffrey D. Ban
Forensic Biology
Section Chief